The blood microbiota in health and disease

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The blood

• Circulation – 5L
• One microliter blood contains:
  ➢ 4-6 million erythrocytes
  ➢ 200-500,000 thrombocytes
  ➢ 4-11,000 leukocytes
I. Methodological challenges, considerations
Challenges when working with blood

• Quick processing time
• Skin contamination
• Catheter contamination
• Sample amount
• Storage
• Anticoagulants
Detection of microbes in blood

- Viability
- Culturable/cultured pathogens only
- Typically monomicrobial detection
- Does not detect viruses
- 50-90% the infectious agent remain unknown
- Takes several days
- Requires large volumes of blood (20-40 ml; molecular/metagenomics studies require 0.5-2 ml)
Methodological considerations for metagenomics on blood samples

• Can detect broad variety of microbes
• Large amount of human DNA
• 16S sequencing
• Shotgun sequencing
Methodological considerations – human background DNA

Song et al., 2014 PLoS ONE 9(7): e103610
Methodological considerations – 16S sequencing

- High throughput
- Fast
- Culture-independent
- Can identify all bacteria

- Species level identification not reliable
- Does not detect fungi/viruses
- Primer biases
Methodological considerations – shotgun sequencing

• Expensive
• Mostly sequencing human genome
• Larger starting material
• Huge amount of data
• Bacteria, fungi, viruses
• Resistance genes

II. Blood microbiota in healthy population
Blood was thought to be sterile

- Viruses / viral segments / phages are often present in healthy people
- High prevalence in the population
- Most often no symptoms present

HHV7 (Guerrero&Bacon)  
TTV (Itoh et al., 2000)
The blood virome

- 8000 participants
- Cell free DNA
- Shotgun sequencing
- ~40% presumed healthy
- ~60% with common disorders (respiratory, cardiovascular, metabolic disorders)

Bacteria in healthy blood?
Are There Naturally Occurring Pleomorphic Bacteria in the Blood of Healthy Humans?

Richard W. McLaughlin,1 Hojatollah Vali,1 Peter C. K. Lau,2 Roger G. E. Palfree,1 Angela De Cicco,1 Marc Sirois,3 Darakhshan Ahmad,4 Richard Villemur,5 Marcel Desrosiers,5 and Eddie C. S. Chan1*

Stenotrophomonas maltophilia

- Electron microscopy
- 16S PCR
- FISH
Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing

Sandrine Païssé,¹* Carine Valle,¹* Florence Servant,¹ Michael Courtney,¹ Rémy Burcelin,¹,²
Jacques Amar,¹,³* and Benjamin Lelouvier¹*

Metagenomics analysis of red blood cell and fresh-frozen plasma units

Pierre Lau,¹,² Samuel Cordey,³ Francisco Brito,⁴ Diderik Tirefort,¹ Thomas J. Petty,⁴
Lara Turin,³ Arthur Guichebaron,¹,² Mylène Docquier,⁵ Evgeny M. Zdobnov,⁴
Sophie Waldvogel-Abramowski,¹ Thomas Lecompte,¹
Laurent Kaiser,³ and Olivier Preynat-Seauve¹,²
III. Blood microbiota in disease
Bacterial translocation can occur in several diseases

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>Method for testing BT</th>
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<tbody>
<tr>
<td>Burn injury</td>
<td>BC, L/M ratio</td>
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<tr>
<td>Trauma/haemorrhagic shock</td>
<td>BC, MLN, endotoxin, L/M ratio</td>
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<tr>
<td>Endotoxin</td>
<td>L/M ratio</td>
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<tr>
<td>Obstructive jaundice</td>
<td>MLN</td>
</tr>
<tr>
<td>Acute pancreatitis</td>
<td>MLN, BC, L/R ratio</td>
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<tr>
<td>Bowel transplant</td>
<td>BC, stool, liver culture</td>
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<tr>
<td>Liver cirrhosis</td>
<td>MLN</td>
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<tr>
<td>Intestinal obstruction</td>
<td>MLN, serosa</td>
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<tr>
<td>Crohn’s disease</td>
<td>MLN, serosa</td>
</tr>
<tr>
<td>Organ donors</td>
<td>MLN, BC, endotoxin</td>
</tr>
<tr>
<td>Elective surgery</td>
<td>MLN, serosa</td>
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<tr>
<td>Aortic aneurysm repair</td>
<td>MLN, serosa</td>
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<tr>
<td>Cardiopulmonary bypass</td>
<td>Endotoxin, L/M ratio</td>
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<tr>
<td>Heart failure</td>
<td>Serum endotoxin</td>
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<tr>
<td>Colchicine overdose</td>
<td>BC</td>
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<tr>
<td>Neutropenia</td>
<td>BC</td>
</tr>
<tr>
<td>Malignancy</td>
<td>BC</td>
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</tbody>
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- Hospitalization time, mortality ↑
- Clinically relevant?
- Prognostic impact?
- Methodology?
- Culture/culture independent?
- Patient/control population?
Detection of microbial DNAemia: does it matter for sepsis management?

Marc J. Struelens

![Graph showing Procalcitonin and 10^6 cells/L levels with positive and negative results.]

**Graph depicting data from:**

Microbes in blood with different underlying conditions

- Heart transplant
- Lung transplant
- Bone marrow transplant
- Pregnancy

- High diversity of phages
- Torque Teno Virus
Blood microbiota in leukemia

Possible solutions

• Remove/decrease human background DNA
• Sequence longer fragments
• Validation of sequencing method
• Filter vs human genome
• Careful selections of controls
  - 16S: measure relative abundances (control vs sample)
  - Shotgun: Phylogeny (control vs sample)
• Optimize 16S PCR

Song et al., 2014 PLoS ONE 9(7): e103610
Summary

• Caution when interpreting data
• Careful selection of controls
• Consideration of appropriate extraction/sequencing methods
• Limited knowledge and evidence of clinical significance

• Pathogen-specific treatment
• Invaluable in the treatment of critically ill / blood culture negative patients
• Patients with unknown etiology
We are faced then with the need for a more intensive investigation of the biological phenomena of stored blood which has been pronounced "sterile." It is clear also that the word "sterile" is not a suitable term in this connexion, because the state of sterility is inconsistent with the presence of vital elements in stored blood.—I am, etc.,

M. R. DRENNAN.

Rehabilitation Centre

Sa.—I exclude a brief memorandum which expresses the general opinion of the members of the British Orthopaedic Association in regard to rehabilitation, and which was drawn up in the names of the members of the Orthopaedic Section of the B.M.A., and which was read at the recent meeting of the Orthopaedic Section of the B.M.A., at Manchester, on March 17, 1942.

The memorandum runs as follows:

The members of the British Orthopaedic Association recognize the value of surgical procedures already adopted by the Ministry of Health in introducing rehabilitation into E.M.S., orthopaedic fractures, and surgical wounds. They are, however, satisfied that the principles of rehabilitation are not widely understood by the public and by the medical profession, and that more education of the public and of the medical profession is urgently needed, in order to ensure the fullest possible rehabilitation for all patients with surgical or orthopaedic injuries. Under the terms of the Town and Country Planning Act, 1932, it is already possible for local authorities to establish special rehabilitation centres, and such an opportunity should be utilized without delay.

B. R. G. CAMERON.

Rehabilitation Centre

Lithotripsy

Sa.—I have used the opportunities on the Lime hill during my years, of Sept. 5 and Oct. 3. A good one made me I can't have too much luck, but there were some confusions in the Lime hill among others who think surgery is different when you talk about. The word said to be a different word, which suggests a difference between the "sterile" and "sterilized" terms—"sterile" in the sense of "sterilized"—and "sterilized" in the sense of "sterile." We are inclined to use the word "sterilized" to indicate the term "sterile." We are inclined to use the word "sterilized" to indicate the term "sterile." Br Med J. 1942 Oct 31; 2(4269): 526.
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